Aqueous Normal Phase ANP HPLC Methods Better to Use Than HILIC Methods - Tech Information

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Why Aqueous Normal Phase (ANP) May Be Considered Superior to HILIC for the Analysis of Polar Compounds

Aqueous Normal Phase (ANP) chromatography has emerged as a powerful alternative to HILIC for laboratories analyzing highly polar compounds, especially when using Cogent TYPE-C™ silica hydride columns.

While HILIC remains useful under certain conditions, ANP overcomes several limitations commonly encountered in HILIC workflows, offering improved speed, stability, flexibility, and method robustness.

Below is a detailed comparison highlighting why ANP is often preferred by technical users who require high precision and versatile selectivity.

Key Advantages of ANP Over HILIC

1. Faster and More Reliable Equilibration

HILIC columns require long equilibration times, typically recommended as 5 minutes per 5 cm of column length, plus additional hold and stabilization time between runs. This can add 12–15+ minutes of overhead before every injection.

By contrast, ANP methods—especially on Cogent TYPE-C columns—equilibrate extremely quickly, requiring about 7 minutes total, even on the same column lengths. Faster equilibration reduces cycle time, increases throughput, and significantly improves method reproducibility.

2. Lower Salt Requirements & Less MS Contamination

To retain some analytes, HILIC often requires high salt concentrations, which:

- Increase mobile-phase preparation difficulty
- Clog ESI nebulizers in LC-MS
- Require frequent instrument cleaning
- Increase baseline noise and suppress ionization

ANP typically uses lower buffer concentrations and achieves strong retention of polar compounds without heavy salt loading, improving MS sensitivity and maintaining cleaner system performance.

3. Superior Retention Time (RT) Precision

HILIC retention can drift because:

- Water migration into the stationary phase shifts the partition layer

- Long re-equilibration creates cumulative variability
- Salt-dependent retention is sensitive to slight composition changes

ANP, on the other hand, produces extraordinarily stable retention times because the retention mechanism is based on predictable interactions at the silica hydride surface.

4. Ability to Retain Polar Compounds at High Aqueous Content

HILIC breaks down when too much water is added to the mobile phase, causing polar compounds to elute early or fail to retain at all.

ANP maintains strong retention even with high levels of aqueous component, allowing method developers to:

- Retain extremely polar compounds
- Run wide gradients
- Adjust mobile-phase composition flexibly without losing control of selectivity

5. Column Lifetime: TYPE-C Columns Are Far More Robust

HILIC columns often have short operational lifetimes, with retention degradation caused by:

- Dissolving silica surfaces
- Salt accumulation
- Changing partition layers

Cogent TYPE-C silica hydride columns demonstrate much longer stability, often lasting up to 10× longer, even under demanding gradient or LC-MS conditions.

6. ANP Uses Conventional Reversed-Phase Mobile Phases

Unlike HILIC, ANP does not require a separate set of HILIC solvents.
ANP uses the same mobile phases used in RP, typically water/acetonitrile with ± acid modifier.

This simplifies:

- Method development
- Solvent management
- Equipment flushing
- Multi-mode workflows
- Lab inventory requirements

7. Dual-Mode Retention for Both Polar and Non-Polar Compounds

Cogent TYPE-C columns can retain polar and non-polar compounds in the same run, thanks to the dual mechanisms:

- ANP retention under high-organic conditions
- Reversed-phase retention under more aqueous conditions

This enables:

- Orthogonal selectivity on a single column
- Broader compound classes in one method
- Fewer column changes

- Simplified screening workflows

This dual capability is demonstrated in separations of polar analytes like metformin and non-polar analytes like glyburide on the same column.

Generic Method Starting Point for Cogent TYPE-C™ Columns

Below is a universally applicable starting protocol for exploring both RP and ANP on TYPE-C columns.

Step 1 — Mobile Phase Selection

Use water and acetonitrile with up to 0.5% formic or acetic acid (TFA acceptable for non-LC-MS applications).

Step 2 — RP Equilibration

Run 6 column volumes at 95% water.

Step 3 — RP Gradient

Run a 20-minute shallow gradient from 95% → 40% water.
For sharper peaks, use a steeper gradient .

Step 4 — ANP Equilibration

Run 100% acetonitrile for ~2 minutes.

Step 5 — ANP Gradient

Run a 20-minute shallow gradient from 90% → 40% acetonitrile.

Step 6 — Compare RP and ANP Data

Evaluate:

- Retention
- Selectivity
- Peak shape
- Elution order

Many mixtures show strong retention in one mode and little in the other—one column may even provide a universal isocratic method for both polar and non-polar components.

Note: Cogent Bidentate C8 and C18 can retain polar compounds even at **100% water**. If applicable, insert a 100% water isocratic run after Step 3.

Selecting the Best TYPE-C Phase

Choose based on analyte polarity mix:

Analyte Type	Recommended TYPE-C Phase
Highly polar, no non-polar components	**Diamond Hydride**
Mixed polarity (polar + non-polar)	**Bidentate C18**
Aromatics / pi-interactions	**Phenyl Hydride**
General use / broad selectivity	**C8, C18**
Hydrophilic functional groups	**Amide**
Traditional normal phase preferences	**Silica-C™**

COGENT
HPLC Columns™

Attachment: COGENT TYPE-C Quick Start Guide Download File

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